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Fracture Toughness of Vocal Fold Tissue: A Preliminary Study

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Abstract

A customized mechanical tester that slices thin, soft samples was utilized to measure fracture toughness of vocal fold tissue. Porcine vocal fold lamina propria was subjected to quasi-static, guillotine-like tests at three equally distanced regions along the anterior-posterior direction. The central one-third where high-velocity collisions between vocal folds occur was found to have the maximum fracture toughness. In contrast, the anterior one-third featured a lower toughness. Fracture toughness can be indicative of the risk of benign and malignant lesions in vocal fold tissue.

Keywords

Vocal folds; Fracture toughness; Benign lesions

1. Introduction

Phonotrauma is believed to be a result of high impact stresses between colliding vocal fold membranes¹. Large stress concentration can cause localized tissue rupture in the mucosal layer, leading to avascular lesions or bleeding. A good understanding of the mechanisms associated with tissue rupture is needed for the etiology of voice disorders. Computational simulations, such as finite element models^{1, 2}, have been used to assess stresses and strains within vocal folds undergoing idealized mechanical vibrations. Conventional mechanical testing methods have been implemented to characterize the elastic properties of vocal fold tissue³. However, its fracture toughness has not yet been investigated. Fracture toughness measures the ability of materials against the propagation of cut or tear, known as “crack” in classical fracture mechanics⁴. Fracture toughness has been measured for biological hard tissues such as bone; however, few attempts have been made to measure this property for soft tissues (e.g., Chu et al.⁵).

There are two common approaches in classical fracture mechanics to study crack propagation in elastic materials, including the stress intensity factor and the energy release

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rate⁴. The stress intensity factor has been defined for linear elastic materials; thus, it is unsuitable for soft tissues, such as vocal fold tissue. The energy approach that considers the required mechanical energy input for damage growth has been employed for soft materials, in which a cutting device slices the specimen⁵. This study pursued the latter approach to measure the fracture toughness of porcine vocal fold lamina propria at three distinct locations along the anterior-posterior direction, which may indicate injury-prone regions. The mechanical toughness can also be a useful parameter for the assessment of biomaterials used for vocal fold regeneration⁶.

2. Methods

2.1 Sample Preparation

Fresh porcine larynges were harvested and their vocal fold tissues were dissected following the protocol used for mechanical testing in Miri et al.⁷. The lamina propria was gently separated from the glottal wall and the muscle. Within three hours postmortem, the samples were snap-frozen while submerging in normal phosphate saline buffer. On the day of the experiment, they were thawed at room temperature. Three distinct regions along the anterior-posterior direction were extracted: one-third anterior (i.e., location A), one-third center (i.e., location B), and one-third posterior (i.e., location C), as shown in Figure 1. These regions were sliced along the coronal plane. In addition, the central region was sliced along the transverse direction. Care was taken to obtain a uniformly small sample thickness, as the methodology required thin samples⁵.

2.2 Experiments

Figure 2 shows a schematic of the test apparatus, which was mounted on a commercial mechanical tester (ElectroForce ELF3200; Bose Inc., Eden Prairie, MN). A polished preconditioned razor blade (0.228 mm thickness; Personna American Safety Razor Company, Cedar Knolls, NJ) was installed into the blade holder, while lubricated to reduce frictions between the blade and sample. The measurements consisted of two successive loading cycles, as schematically shown in Figure 3. The blade velocity was 0.1 mm/s to enforce quasi-static conditions (the total time was less than 5 minutes for each sample). In the first cycle, the moving blade cut the tissue, and then a second cycle was performed to estimate the friction force between the blade and the tissue sample. The difference of the projection areas under the two curves yielded the fracture toughness, as highlighted in Figure 3. The tissue hydration was maintained by adding water drops to the sample surface since the testing apparatus did not allow submerging samples in a bath solution. The blade lubrication was also preserved using oil droplets. The blade position was adjusted to make a 4–6 mm deep cut in tissue samples to assure the estimation of the fracture properties (see below and Taylor et al.⁴). The beginning of the cut (up to 1 mm in most cases) is probably dominated by elastic deformation; hence, a longer cut is suggested to reduce the contribution of elastic deformation. In the present study, the error associated with elastic deformation was less than 10%.

2.3 Data analysis

The lubricated, guillotine-like, cutting system not only provided controlled crack propagation under Mode I⁴, but also eased the use of small-sized samples. Figure 3 displays the possible deformation mechanisms occurring within small tissue samples under the blade movement. After the first contact between the blade and sample, the tissue undergoes nearly-elastic deformation and the blade tip acts like a conical indenter. The stress concentration leads to the first cut, which reduces the tissue resistance against further cuts. The load-time history consists of a series of elastic deformation, cutting, and force relaxation. The fracture toughness is determined from the mechanical energy W consumed by the blade tip. The mechanical energy calculated from the area under the displacement-force curves yields the fracture toughness, i.e.,

$$J = \int F dx / (hL) \quad (1)$$

where F denotes the normal force at the crack tip, h is the tissue thickness, and L is the crack length. The parameter J that represents the energy absorbance of the tissue for high stress concentrations is defined as fracture toughness. Comparisons within each group were performed using two-tailed, paired t -tests.

To present the empirical relation in Eq. (1), one may extend the energy balance between the blade and the tissue as (in the form of differentials)

$$Jhdx + d(\delta u) + F_f dx = F_r dx + dU \quad (2)$$

where F_r denotes resistance force imposed by tissue on the blade, F_f represents friction force on the blade, U represents internal strain energy in the tissue, and δu is the change in the stored internal recoverable strain energy potential. The strain energy changes are mainly associated with the elastic deformation at the beginning of the cut. Ignoring the minimal changes in the internal strain energy, one may write the energy balance between the blade and the tissue as

$$Jhdx = (F_r - F_f)dx, \quad (3)$$

in which the fracture toughness J is defined as a constant (i.e., mean) value for each case, and the net force in Eq. (3) is equal by F in Eq. (1).

3. Results

3.1 Physical model

The possible dependency of the fracture toughness on sample thickness was investigated using silicone rubber samples that were cured by mixing equal ratios of Dragon Skin® constituents (model 10 medium; Smooth-On Inc., PA) and Silicone Thinner (Smooth-On Inc.). This composition exhibited a similar Young's modulus as that of porcine vocal fold lamina propria⁷. The fracture toughness values of the silicone rubber were consistent (420–440 J/m²) when the sample thickness was varied between 0.3 and 1 mm. The toughness values significantly reduced for greater thicknesses (e.g., 300 J/m² for 2 mm). The tissue

samples used in this study ranged in thickness from 0.3 to 0.6 mm, a typical size of porcine lamina propria, which ensures the consistency of the current measurements. The results in each group (see Table 1) had smaller standard deviations than those of other mechanical properties, such as Young's modulus⁷. One important fact about the experiments was to mount the sample with a minimal pre-tension that assured the flatness of the samples.

3.2. Vocal fold tissue

The results obtained for seven vocal fold samples (in each test group) are summarized in Table 1 (see also Fig. 4). The central region, where high-amplitude vibrations were observed during self-sustained oscillations in excised larynx experiments⁸, exhibited the highest fracture toughness. This region is also believed to undergo maximum impact stress concentrations², in agreement with experimental observations⁸. The lowest toughness value was for location A (p -value of 0.007, when compared to those of location B). The fracture toughness is a scalar quantity; however, it can depend on the direction of the crack propagation or blade cut. The tissue response for cuts along the anterior-posterior direction (169.4 J/m^2) was significantly (p -value of 0.0005) smaller than that of the perpendicular direction (451.1 J/m^2).

4. Discussion and concluding remarks

The experimental concerns in the present work included several factors. The loading rate was designed to impose quasi-static loading that minimized dynamic effects. The loading period was also greater than the local relaxation time of the samples. In addition, a small pre-tension in the tissue ensured passage of the blade through the first cut and satisfaction of test conditions for Mode I fracture toughness determination. The selected minimum of the crack length (3 mm) allowed three divisions along the vocal fold length. The load-displacement histories associated with the three regions are shown in Fig. 4. To have a better comparison of the data, the force values were divided to the sample thickness (h) and the displacement values were normalized to the crack length (L).

The variation of the elastic modulus along the anterior-posterior direction has been investigated for vocal fold tissue^{3, 9}. For example, the indentation moduli displayed a positive gradient from the center toward the anterior direction. Hence, the anterior region has a higher elasticity (corresponding to a greater resistance at the beginning of the slicing in Fig. 4) and a lower toughness (corresponding to a smaller projection area in Fig. 4). In terms of engineering mechanics, this region shows a more brittle behavior. The lower toughness values may explain the increased probability of occurrence of benign lesions in the anterior region². Because the central region (i.e., location B) involves a high fraction of vocal fold oscillation⁸, it has a high possibility of crack formation at micro-scale. The impact-induced cracks should be healed quickly to preserve integrity and functions of the vocal fold tissue. Thus, the central region should exhibit a high capacity for the absorbance of deformation-induced energy, in analogy with its high toughness values. In terms of engineering mechanics, this region shows a more ductile behavior. Finally, the posterior region with a mechanical behavior between the first two regions involves less likelihood of damage and injuries.

The variations of physical properties, such as stiffness and fracture toughness, along the anterior-posterior direction are dictated by the tissue microstructure^{3, 10}. An organization of elastic fibers, such as elastin and collagen, provides tissue stiffness and its extent of deformation¹⁰. In contrary, high-damping extracellular matrix components, such as hyaluronic acids and glyco-samino-glycans, mainly contribute to tissue flexibility. The higher toughness values may suggest a greater fraction of high-damping components in the central region. The microstructure-toughness relations may justify the differences between the results of coronal cuts and those of transverse cuts.

The transverse direction was parallel to the dominant orientation of collagen fibrils. The difference between the toughness values of coronal and transverse slicing highlights the tissue anisotropy and the contribution of collagen fibrils (see Miri et al.⁷). The longitudinal direction has the strongest stiffness compared to other directions because of the spatial orientation of collagen fibrils^{3, 10}. Therefore, tissue exhibits its highest toughness when sliced in the coronal plane. Gunter² also showed that dynamic shear stresses reach their maximum values in the anterior region, location A in Figure 1. The vocal fold toughness values for coronal slicing are similar to those of skin. Among the fracture toughness values for various soft tissues measured by Pereira et al.¹¹, the abdominal skin appears to have a similar toughness than vocal folds.

The fracture toughness of the porcine vocal fold was found to vary non-uniformly along the posterior-anterior direction, reaching its maximum in the central region. The anterior region exhibited a low toughness. Despite physiological differences between human and porcine vocal folds, this fact perhaps might explain clinical observations that the anterior region has the highest likelihood of phonotrauma. This study may provide novel insights into mechanical factors associated with the development of vocal fold lesions. This methodology constitutes a step towards the development of a characterization tool that can evaluate the fracture toughness of vocal fold tissue. In addition to existing parameters, such as elastic modulus, the fracture toughness may provide a design criterion for injectable biomaterials⁶. The fracture toughness includes elasticity, hysteresis, flexibility and integrity of the vocal fold tissue; thus, it would give a superior factor to assess engineering scaffolds. The measurement of the fracture toughness is an invasive method and cannot be used for in-vivo applications. This fact prevents the use of this methodology for clinical situations.

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Figure 1.
Locations A, B, and C are depicted in a porcine vocal fold sample.

Figure 2.
Schematics of the fracture-toughness set-up, adopted from Chu et al.⁵.

Figure 3. Typical loading-unloading curves for a soft tissue sample subjected to fracture testing. The shaded area gives the fracture toughness.

Figure 4.
Force-displacement histories (i.e., the first two cycles) for four selected samples.

Table 1

Fracture toughness values (J/m^2) for porcine lamina propria from coronal cutting at three locations and transverse cutting in the centre ($m = 7$ for each group).

Location (plane) <i>p</i> -value	A (coronal) 0.007	B (coronal) ---	B (transverse) 0.0005	C (coronal) 0.003
<i>J</i> (J/m^2)	266.1 ± 72.6	451.1 ± 87.9	169.4 ± 30.1	333.9 ± 82.4